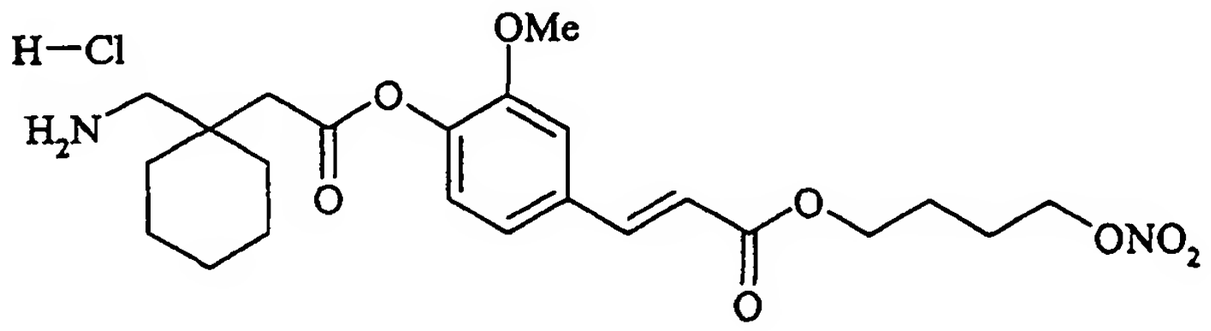
preferred precursor drugs are the following: gabapentine, norvaline, arginine, pregabaline, (S)3-isobutylGABA, agmatine.

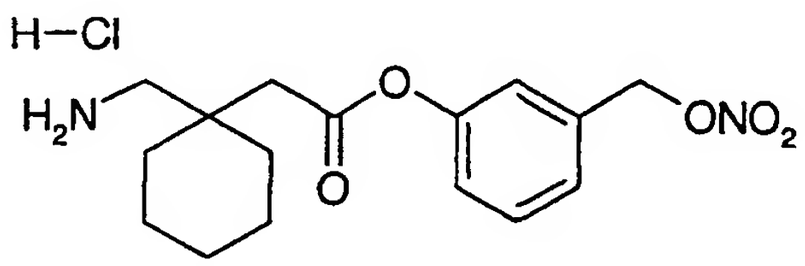
The preferred compounds of formula (I) according to the present invention are the following:

1-(aminomethyl)cyclohexan acetic acid 2-methoxy-4-[(1E)-3-[4-(nitrooxy) butoxy]-3-oxy-1-propenyl]phenyl hydrochloride ester (XV)



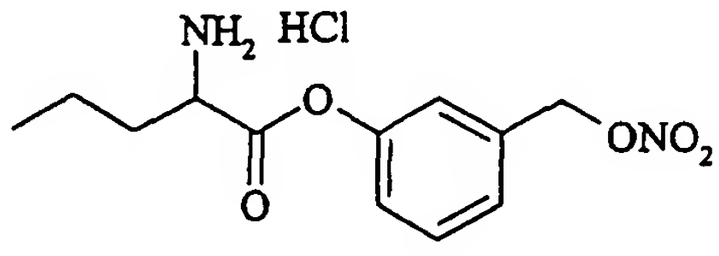
(XV)

1-(aminomethyl)cyclohexan acetic acid 3-(nitrooxymethyl) phenyl hydrochloride ester (XVI)



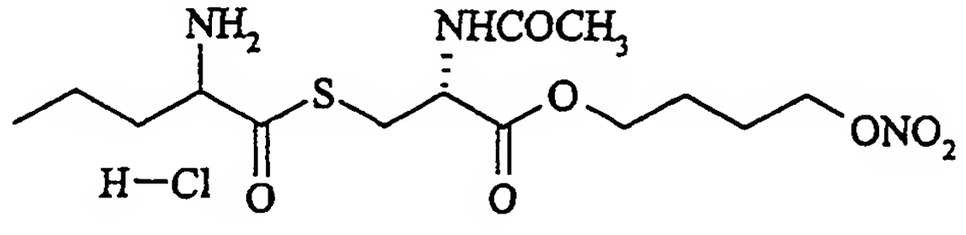
(XVI)

2-aminopentanoic acid 3-(nitrooxymethyl)phenyl hydrochloride ester (XVII)



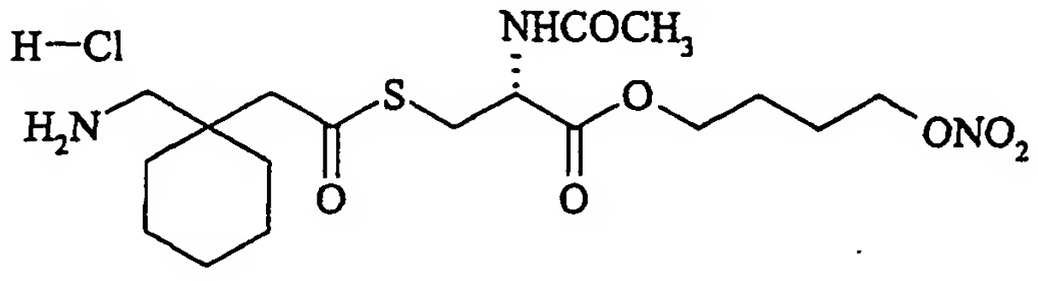
(XVII)

(S)-N-acetylcysteine-, 4-(nitrooxy)butyl ester, 2-amino hydrochloride pentanoate (XVIII)



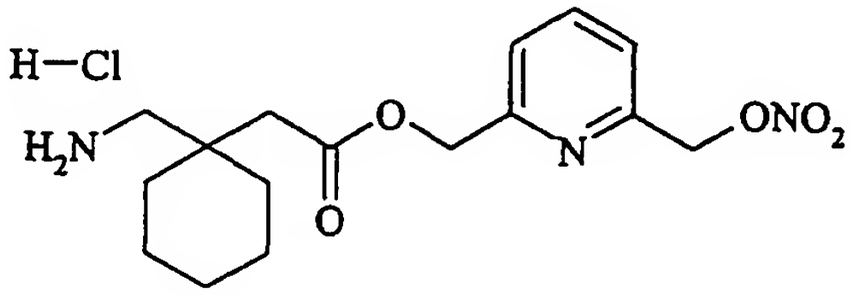
(XVIII)

(S)-N-acetylcysteine-, 4-(nitrooxy)butyl ester, 1-(aminomethyl)cyclohexanacetate hydrochloride (XIX)



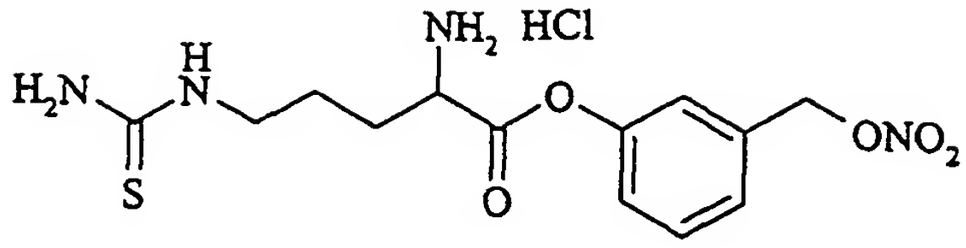
(XIX)

1-(aminomethyl)cyclohexanacetic acid-, [6-(nitrooxy methyl)-2-pyridinyl]methyl hydrocloride ester (XX)



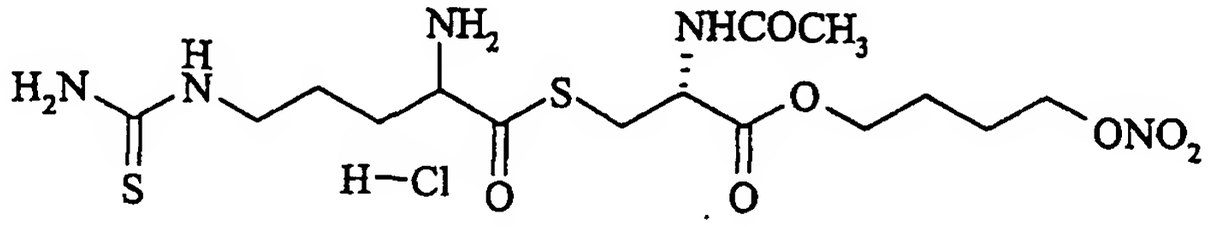
(XX)

alpha-amino-delta-thioureidopentanoic acid, 3-(nitrooxy methyl)phenyl hydrocloride ester (XXI)



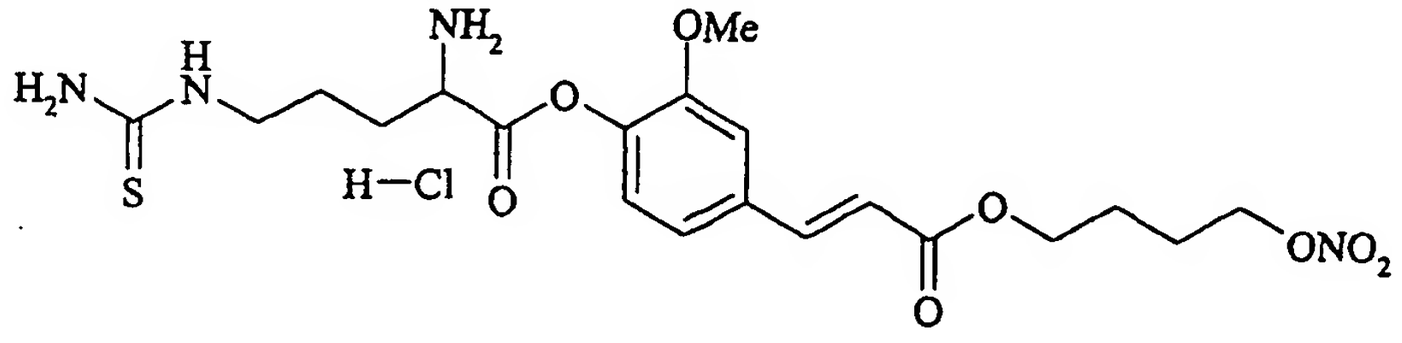
(XXI)

(S)-N-acetylcysteine-, 4-(nitrooxy)butyl ester, alpha-amino-delta-thioureidopentanoate hydrocloride (XXII)



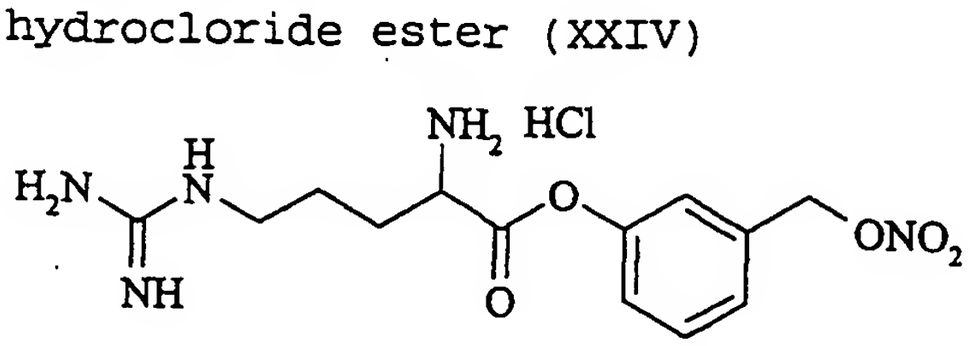
(XXII)

alpha-amino-delta-thioureidopentanoic acid, 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl]phenyl hydrocloride ester (XXIII)



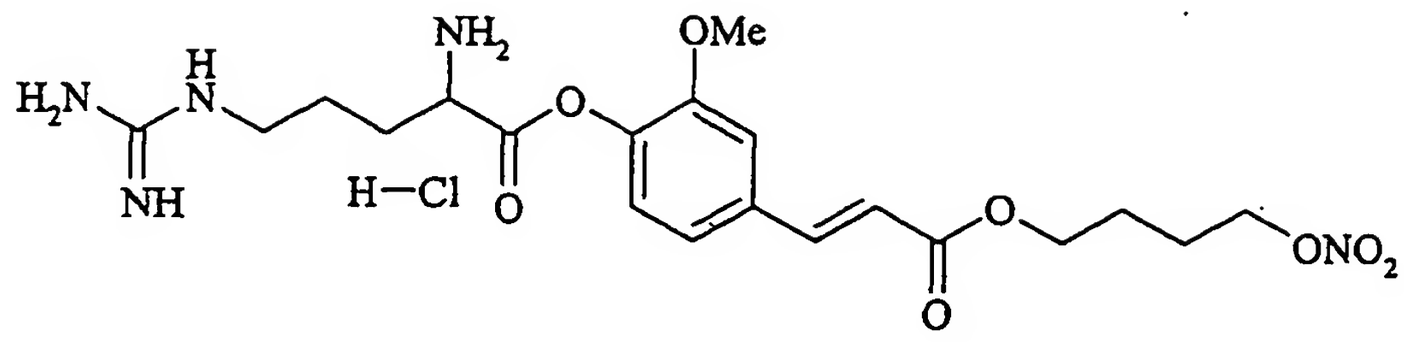
(XXIII)

2-amino-5-guanidinopentanoic acid, 3-(nitrooxy methyl)phenyl hydrocloride ester (XXIV)



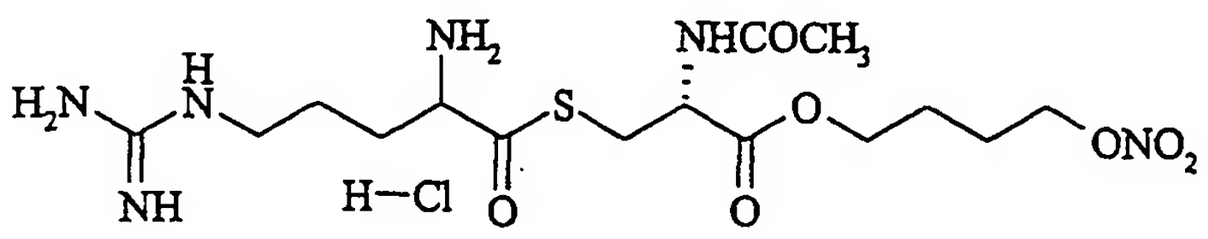
(XXIV)

2-amino-5-guanidinopentanoic acid-, 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl]phenyl hydrocloride ester (XXV)



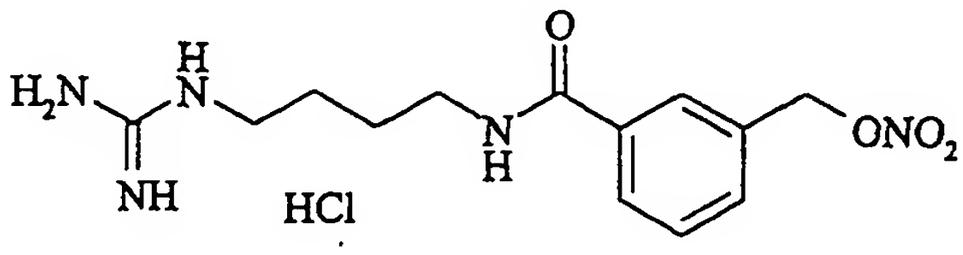
(XXV)

(S)-N-acetylcysteine-4-(nitrooxy)butyl ester, 2-amino-5-guanidinopentanoate hydrocloride (XXVI)



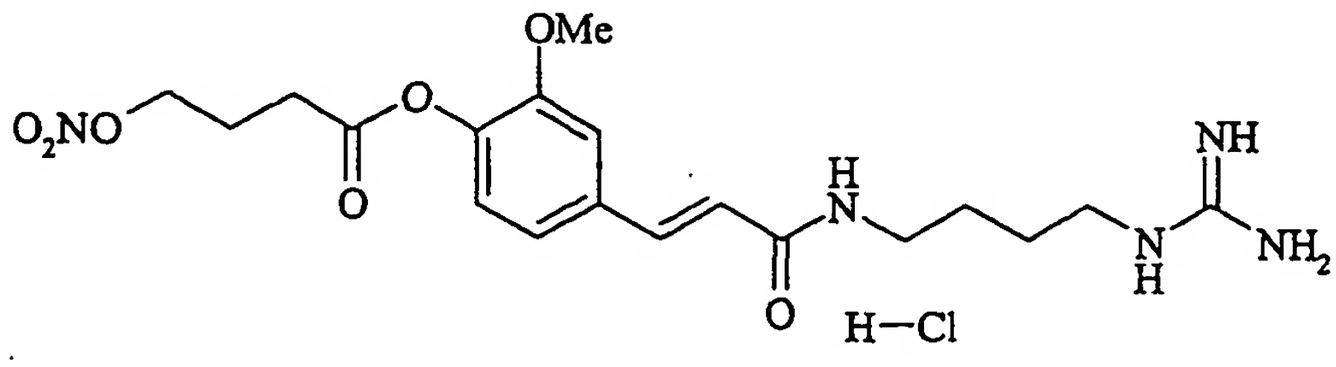
(XXVI)

4-(guanidine)butyl-3-nitrooxymethylbenzamide (XXVII)



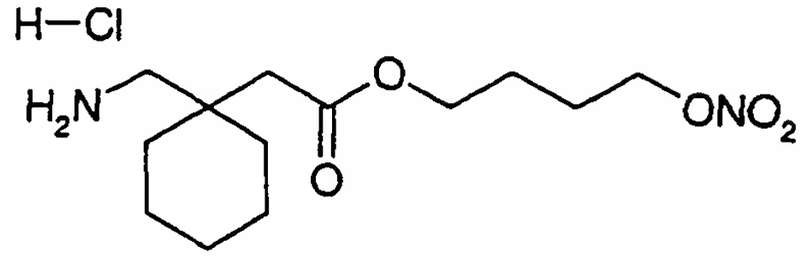
(XXVII)

4-(guanidine)butyl-3-[4-(4'-nitrooxybutyryloxy)-3-(methoxy)]phenyl-2-propenamide chloride (XXVIII)



(XXVIII)

1-(aminomethyl)cyclohexan acetic acid 4-(nitroxy)butyl hydrochloride ester (XXIX)



(XXIX)

The preferred above compounds with formulas (XV) to (XXIX) can be used as nitrate salts.

The compounds according to the present invention, when they contain in the molecule one salifiable nitrogen atom, can be transformed into the corresponding salts by reaction in organic solvent such for example acetonitrile, tetrahydrofuran with an equimolar amount of the corresponding organic or inorganic acid.

Examples of organic acids are: oxalic, tartaric, maleic, succinic, citric acid.

Examples of inorganic acids are: nitric, hydrocloric, sulphuric, phosphoric acid.

Nitrate salts are preferred.

The compounds of the invention have shown to have an improved activity in the chronic pain treatment, in particular neuropathic, both at the central and peripheral nervous system level. Besides it has been surprisingly found by the Applicant that the invention compounds not only have an improved efficacy in reducing the neuropathic pain, but unexpectedly show also a lowering action of the progress of the pathological condition which causes the neuropathic pain. For example when the drugs of the present invention are administered to diabetic patients for reducing the diabetic neuropathic pain, it has been found that they are able not

only to reduce neuropathies, but also to reduce the complications caused by diabetes, for example affecting the blood vessels and/or the renal apparatus.

The compounds of the invention are particularly effective in the neuropathic pain treatment, for example the diabetic neuropathic pain, the post-infarct pain.

The compounds of the invention can also be used in combination or in admixture with NO-donor compounds of the prior art.

Said compounds contain for example in the molecule one or more ONO_2 or ONO groups.

The NO-donor compounds which can be used in combination with the invention compounds must comply with the test in vitro defined hereinafter.

The test relates to the nitric oxide generation from the NO-donors, for example nitroglycerin, niocorandil, nitroprussiate, etc., in the presence of endothelial cells (method a) or platelets (method b).

a) Endothelial cells

Human cells of the umbilical vein, cultured on plates, having a 10^3 density cells/well were incubated for 5 minutes with scalar concentrations of NO-donor (1-100 µg/ml). The incubation medium (physiologic solution, for example Tyrode) was then analyzed to determine the capability to generate NO of the compound under test, by means of:

1) nitric oxide detection by chemiluminescence;

2) cGMP determination (cyclic GMP n° 2715 of the above mentioned Merck).

For the analysis by chemiluminescence, an amount equal to 100 µl was injected in the reaction chamber of a chemiluminescence analyzer containing glacial acetic acid and potassium iodide. The nitrites/nitrates present in the medium, under these conditions are converted into NO which is then detected after reaction with ozone, wich produces light. In the equipments which measure the chemiluminescence, the luminescence produced is directly proportional to the generated NO levels and can be measured by a suitable photomultiplying unit of a chemiluminescence analyzer. The photomultiplier converts the incident light in electric voltage, which is quantitatively recorded. On the basis of a calibration curve, prepared with scalar nitrite concentrations, it can be quantitatively determined the generated NO amount. For example, from the incubation of 100 µM of nicorandil, an amount equal to about 10 µM of NO was generated.

For cGMP determination, an aliquot of the incubation medium (equal to 100 µl) was centrifuged at 1,000 revolutions per 20 seconds. The surnatant was removed and the sediment treated with iced phosphate buffer (pH 7.4). The cGMP levels produced were tested by specific immuno-enzymatic reactants. From said experiments it resulted that, under these experimental conditions, the incubation with one of the various tested NO-donors caused a significant increase of cGMP with respect to the values obtained in absence of a NO-donor. For example, after an incubation with 100 µM of sodium nitroprussiate, an increase of about 20 times the value obtained with the

incubation of the carrier alone without NO-donor was recorded.

b) Platelets

Washed human platelets, prepared substantially in the same way as described by Radomski et al, (Br. J. Pharmacol. 92, 639-1987), were used. 0.4 ml aliquots were incubated with NO-donor scalar concentrations (1-100 µg/ml) for 5 minutes. The incubation medium (for example Tyrode) was then analyzed to determine the capability of the tested compound to generate NO, by determination of nitric oxide by chemiluminescence and the cGMP determination, as described in the previous paragraph for the same analyses carried out on endothelial cells. For the determination by chemiluminescence, also in this case, on the basis of a calibration curve prepared with scalar nitrite concentrations, it was possible to quantitatively determine the produced NO amount. For example, after an incubation of 100 µM of nicorandil, an amount equal to 35 µM of NO was generated.

For cGMP determination, it resulted that also in these experimental conditions the incubation with one of the tested NO-donors gave a significant increase of cGMP with respect to the values obtained in absence of a NO-donor. For example, after an incubation with 100 µM of sodium nitroprussiate, an increase of about 30 times the value obtained with the incubation of the only carrier without NO-donor, was recorded.

The preferred NO-donor compounds are those containing in the moelcule radicals of drugs belonging to the classes of aspirin, ibuprofen, paracetamol, naproxen, diclofenac,

flurbiprofen. The syntheses of these preferred compounds are described in patent applications WO 95/30641, WO 97/16405, WO 95/09831, WO 01/12584.

The compounds of the invention can be obtained by the methods described hereafter.

If in the drug molecule more reactive groups such for example COOH and/or HX are present, they must be usually protected before the reaction according to the methods known in the prior art; for example as described in the volume by Th. W. Greene: "Protective groups in organic synthesis", Harward University Press, 1980.

The acylhalides are prepared according to the methods known in the prior art, for example by thionyl or oxalyl chloride, halides of P^{III} or P^{V} in solvents inert under the reaction conditions, such as for example toluene, chloroform, DMF, etc.

1) When in formula (I) b0 = 0 and the free valence of the radical R of the drug is saturated with a carboxylic group, the synthesis methods to obtain the corresponding nitrooxyderivatives are the following:

1.A) The drug of formula RCOOH is treated with an agent activating the carboxyl group selected from N,N'carbonyldi imidazol (CDI), N-hydroxybenzotriazol and dicyclohexylcarbodiimide (DCC) in solvent such as for example DMF, THF, chloroform, etc., at a temperature in the range from -5°C to 50°C and reacted in situ with a compound HO-Y-Hal, wherein Y and Hal are as above defined.

$$RCOOH \xrightarrow{DCC,\ HO\text{-}Y\text{-}Hal} R\text{-}CO\text{-}O\text{-}Y\text{-}Hal \qquad (1C)$$

1.B) In alternative, the drug acylhalide is reacted with a compound HO-Y-R_{8A}, wherein Y is as above, R_{8A} is OH or a halogen in the presence of a base, in an organic solvent inert under the reaction conditions according to the scheme below reported:

$$RCOHal + HO\text{-}Y\text{-}R_{8A} \dashrightarrow R\text{-}COO\text{-}Y\text{-}R_{8A} \quad (1D)$$

1.C) When the compounds obtained in the above reactions have formula R-COO-Y-Hal the corresponding nitrooxyderivatives are obtained by reacting the compound R-CO-O-Y-Hal with $AgNO_3$ in organic solvent such as acetonitrile, tetrahydrofuran according to the scheme:

$$R\text{-}COO\text{-}Y\text{-}Hal + AgNO_3 \dashrightarrow R\text{-}COO\text{-}Y\text{-}ONO_2$$

1.D) When the compounds obtained in the above reactions have formula R-COO-Y-OH the hydroxyl group is subjected to halogenation, for example with PBr_3, PCl_5, $SOCl_2$, PPh_3 + I_2, and then reacted with $AgNO_3$ in organic solvent such as acetonitrile, tetrahydrofuran.

2) When in formula (I) b0 = 0, and the reactive function of the drug is the group NH_2, the synthesis methods to obtain the corresponding nitrooxyderivatives are the following:

2.a) By reaction of the drug R-NH_2 with an acyl halide of formula Hal-Y-COHal, wherein Y and Hal are as above, according to the scheme:

$$R\text{-}NH_2 + Hal\text{-}Y\text{-}COHal \dashrightarrow R\text{-}NHCO\text{-}Y\text{-}Hal \quad (2A)$$

2.b) By reaction of the drug R-NH_2 with an acyl halide of formula OH-Y-COHal, wherein Y and Hal are as above, according to the scheme:

$$R\text{-}NH_2 + Hal\text{-}Y\text{-}COCl \dashrightarrow R\text{-}NHCO\text{-}Y\text{-}OH \quad (2B)$$

2.c) When the compounds obtained in the above reactions have formula R-NHCO-Y-Hal or R-NHCO-Y-OH the corresponding

nitrooxyderivatives are obtained as above described in 1.C and 1.D respectively.

3. When in formula (I) b0 = c0 = 1, and the free valence of the radical R of the drug is saturated with a carboxylic group, the synthesis methods to obtain the corresponding nitrooxyderivatives are the following:

3.a) Alternatively, the acyl halide of the drug and the compound of formula $HX-X_2-COOH$, wherein X and X_2 are as above, are reacted according to the methods known in the prior art, to give the compound $R-CO-X-X_2-COOH$ which is transformed into the corresponding sodic salt and reacted with a compound of formula $Hal-Y-R_8$ wherein Hal and Y are as above and R_8 is Cl, Br, Iodine, OH:

$$R-COHal + HX-X_2-COOH \dashrightarrow R-CO-X-X_2-COOH \quad (3.A)$$

$$R-CO-X-X_2-COONa + Hal-Y-R_{8A} \dashrightarrow R-CO-X-X_2-CO-Y-R_{8A} \quad (3.A')$$

When R_{8A} = OH the compound of formula (3.A') is subjected to halogenation as above described in 1.D; when R_{8A} = Hal the compound of formula (3.A') is reacted with $AgNO_3$ in organic solvent such as acetonitrile, tetrahydrofüran:

3.b) When Y_T is a C_4 linear alkylene, the precursor of B of formula $HO-X_2-COOH$ is reacted with triphenylphosphine in the presence of a halogenating agent such as CBr_4 or N-bromosuccinimide in tetrahydrofuran to give the compound of formula $HO-X_2-COO(CH_2)_4Br$ which is reacted with the molecule of the drug RCOOH as described in 1.A and 1.C.

4) When in formula (I) p = 1 b0 = c0 = 1, and the reactive function of the drug is the group NH_2, the synthesis methods to obtain the corresponding nitrooxyderivatives are the following:

4.a) Reaction of the drug R-NH_2 with an acyl halide of formula HX-X_2-COHal, wherein X and X_2 are as above, according to the methods known in the prior art, to give the compound R-NH-CO-X_2-XH which is reacted with a compound of formula R_{8A}-Y-COHal wherein R_{8A} and Y are as above.

$$R\text{-}NH_2 + HX\text{-}X_2\text{-}COCl \dashrightarrow R\text{-}NH\text{-}CO\text{-}X_2\text{-}XH \quad (4.A)$$

$$R\text{-}NH\text{-}CO\text{-}X_2\text{-}XH + R_{8A}\text{-}YCO\text{-}Hal \dashrightarrow R\text{-}NH\text{-}CO\text{-}X_2\text{-}X\text{-}CO\text{-}Y\text{-}R_{8A} \quad (4A')$$

4.b) Alternatively, the drug R-NH_2 is reacted with a compound of formula HX-X_2-COOH, wherein X and X_2 are as above, in the presence of dicyclohexylcarbodiimide as described in 1.A, to give the compound R-NH-CO-X_2-XH, which is reacted with a compound of formula R_{8A}-Y-COCl wherein R_{8A} and Y are as above defined, to give the following compound: R-NH-CO-X_2-X-CO-Y-R_{8A} (4.B)

When R_{8A} = OH the compound of formula (4.B) or of formula (4a') is subjected to halogenation as above described in 1.D; when R_{8A} = Hal the compound of formula (4.B) is reacted with $AgNO_3$ in organic solvent such as acetonitrile, tetrahydrofuran.

When the compounds of the present invention have one or more chiral centres, they can be used in a racemic form, as mixtures of diastereoisomers or enantiomers, as single enantiomers or single diastereosisomers. If the compound shows geometric asymmetry, the compound in the cis or trans form can be used.

The compounds object of the present invention are formulated in the corresponding pharmaceutical compositions for pareneteral, oral and topical use according the techniques well known in the field, together with the usual excipients,

see for example the volume "Remington's Pharmaceutical Sciences 15th Ed."

The amount on a molar basis of the active principle in said formulations is the same or lower than the maximum posology indicated for the precursor drugs. Also higher doses can be used, considering their very good tolerability.

The daily administrable doses are those of the precursor drugs, or in case lower. The daily doses can be found in the publications of the field, such as for example in "Physician's Desk reference".

A further object of the invention is the use of analgesic drugs for the treatment of the chronic pain, in particular the neuropathic pain, in combination with NO-donor compounds as above defined.

The radicals of the conventional analgesic drugs for the chronic pain have been indicated above with R wherein the free valence is saturated with T_{1A}, wherein $T_{1A} = COZ_1$ wherein Z_1.= SH, OZ, NHR_{1C}, XZ, wherein Z, R_{1C} and X are as above defined.

Said compounds of formula $RCOZ_1$ are the precursor drugs of R.

It has been found that the combination of the precursor drugs of R in combination with the NO-donor compounds shows a synergic effect, whereby it is possible to use a lower amount of the analgesic compound for the chronic pain, whereby the side effects are reduced.

Besides the above mentioned precursor drugs of R, the following ones can be mentioned: lamotrigine, topiramate, tiagabime, zonisamide, carbamazepine, felbamate, amineptine, amoxapine, demexiptiline, desipramine, nortriptyline, opipramol, tianeptine, amitriptyline, butriptyline,

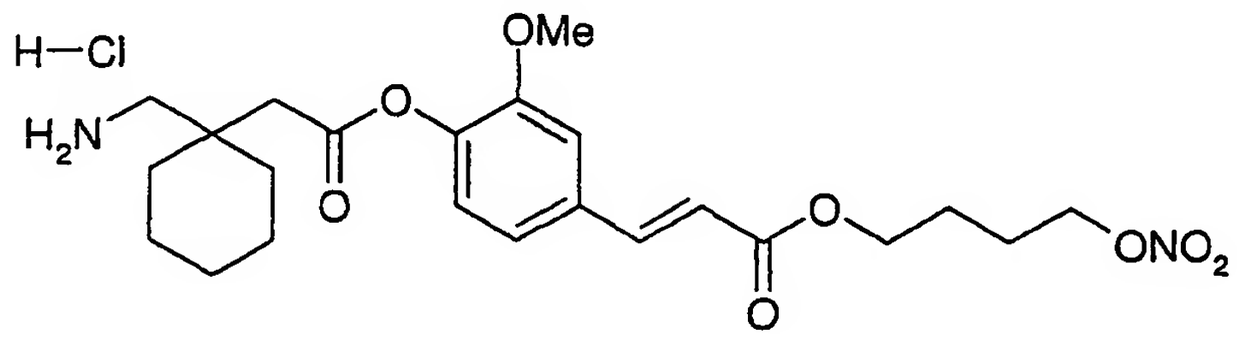
clomipramine, dibenzepin, dimetacrine, dothiepin, doxepin, fluacizine, imipramine, iprindole, lofepramine, melitracen, noxiptilin, propizepine, protriptyline, trimipramine.

The NO-donor compounds are as above defined.

The following Examples illustrate the invention without limiting the scope thereof.

EXAMPLE 1

Synthesis of the 1-(aminomethyl)cyclohexan acetic acid 2-methoxy-4-[(1E)-3-[4-(nitrooxy) butoxy]-3-oxy-1-propenyl] phenyl hydrochloride ester (XV)



A) Synthesis of the 1-(N-tert-butoxycarbonylaminomethyl) cyclohexan acetic acid

To a solution of 1-(aminomethyl)cyclohexanacetic acid (10 g, 58.4 mmoles) in a mixture of dioxane (100 ml) and water (150 ml), triethylamine (16.27 ml, 116.8 mmoles) and di-tert-butyldicarbonate (15.3 g, 70 mmoles) are added. The reaction mixture is left at room temperature, under stirring for 4 hours. After the solution has been cooled to 0°C it is brought to pH 2 with HCl 5%. The precipitate is filtered and dried under vacuum. 15 g of the expected product are obtained as a white solid having m.p. = 125°-127°C

B) Synthesis of 2-methoxy-4-[(1E)-3-[4-(bromo)butoxy]-3-oxy-1-propenyl]phenol

To a solution of ferulic acid (11.6 g, 59.7 mmoles) in tetrahydrofuran (400 ml) tetrabromomethane (39.62 g, 119.47 mmoles) and triphenylphosphine (31.34 g, 119.47 mmoles) are added. The obtained mixture is kept under stirring at room temperature for 5 hours, filtered and evaporated at reduced pressure. The crude residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 7/3. 8 g of the expected compound are obtained as a yellow solid having m.p. = 86°-89°C

C) Synthesis of 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl]phenol

To a solution of 2-methoxy-4-[(1E)-3-[4-(bromo) butoxy]-3-oxy-1-propenyl]phenol (8 g, 24.3 mmoles) in acetonitrile (500 ml) silver nitrate (12.25 g, 72.9 mmoles) is added. The reaction mixture is heated at 40°C for 12 hours sheltered from light. The formed salt is removed by filtration and the solution is evaporated at reduced pressure. The residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 75/25. 4 g of the expected product are obtained as a yellow solid having m.p. = 65°-68°C

D) Synthesis of the 1-(N-tert-butoxycarbonylaminomethyl) cyclohexan acetic acid 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl]phenyl ester

To a solution of 1-(N-tert-butoxycarbonyl aminomethyl)cyclohexan acetic acid (2.5 g, 9.2 mmoles) in chloroform (200 ml) and N,N-dimethylformamide (3 ml), 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl] phenol (3.15 g, 10.1 mmoles), dicyclohexylcarbodiimide (5.7 g, 27.6 mmoles) and N,N-dimethylaminopyridine (33 mg, 0.27 mmoles) are added.

The reaction mixture is left at room temperature for 3 hours under stirring, filtered and evaporated at reduced pressure. The obtained residue is treated with ethyl acetate and washed with water. The organic phase is dried with sodium sulphate and evaporated at reduced pressure. The residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 9/1. 5 g of the expected compound are obtained as an oil.

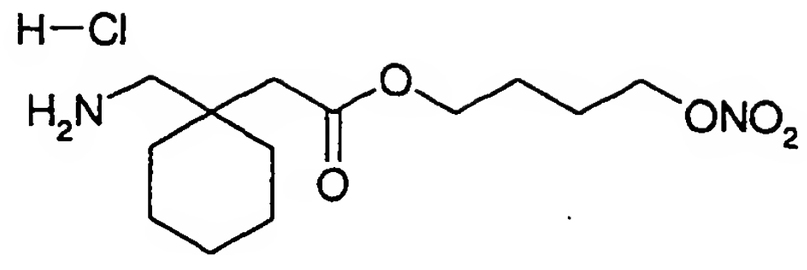
E) Synthesis of the 1-(aminomethyl)cyclohexan acetic acid 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl] phenyl hydrochloride ester

To a solution of 1-(N-tert-butoxycarbonylamino methyl)cyclohexan acetic acid 2-methoxy-4-[(1E)-3-[4-(nitrooxy) butoxy]-3-oxy-1-propenyl]phenyl ester (5 g, 8.8 mmoles) in ethyl acetate (100 ml) a solution HCl 1N in ethyl acetate (50 ml) is added. The reaction mixture is left overnight at room temperature, then concentrated under vacuum to a volume of 40 ml. The obtained residue is treated with ethyl ether. The precipitate is filtered and dried under vacuum. 1.8 g of the expected compound are obtained as a white solid having m.p. = 103°-105°C.

^{1}H-NMR ($CDCl_3$) ppm: 8.43 (2H, m); 7.55 (1H, d); 7.10 (3H, m); 6.34 (1H, d); 4.51 (2H, t), 4.26 (2H, t); 3.89 (3H, s); 3.12 (2H, s); 2.81 (2H, s); 1.82 (4H, m); 1.54 (10H, m).

EXAMPLE 2

Synthesis of the 1-(aminomethyl)cyclohexan acetic acid 4-(nitrooxy)butyl hydrochloride ester



A) Synthesis of the 1-(N-tert-butoxycarbonylaminomethyl) cyclohexan acetic acid 4-(bromo)butyl ester

To a solution of 1-(N-tert-butoxycarbonyl aminomethyl)cyclohexan acetic acid (1 g, 3.6 mmoles) in N,N-dimethyl formamide (50 ml) cooled at 0°C, sodium ethylate (246 mg, 3.6 mmoles) is added.

The reaction mixture is left at 0°C under stirring for 30 minutes and then 1,4-dibromobutane (1.28 ml, 10.8 mmoles) is added. The solution is left under stirring overnight at room temperature, then diluted with ethyl ether and washed with water. The organic phase dried with sodium sulphate is evaporated under vacuum. The obtained residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 8/2. 0.7 g of the expected compound are obtained as an oil.

B) Synthesis of the 1-(N-tert-butoxycarbonylaminomethyl) cyclohexan acetic acid 4-(nitrooxy)butyl ester

To a solution of 1-(N-tert-butoxycarbonylamino methyl)cyclohexan acetic acid 4-(bromo)butyl ester (1 g, 2.5 mmoles) in acetonitrile (200 ml), silver nitrate (1.3 g, 7.5 mmoles) is added. The reaction mixture is heated at 80°C for 6 hours sheltered from light. The formed salt is removed by filtration and the solution is evaporated at reduced pressure. The residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 8/2. 0.8 g of the expected compound are obtained as an oil.

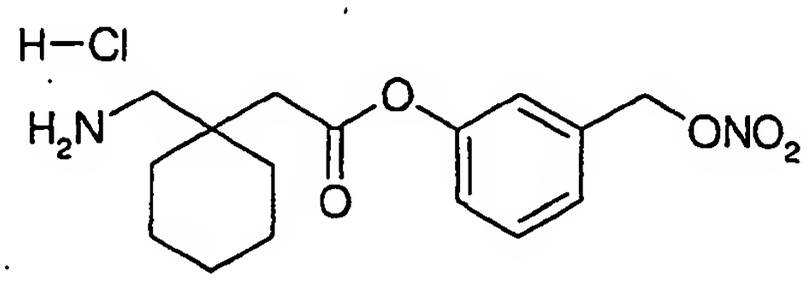
C) Synthesis of the 1-(aminomethyl)cyclohexan acetic acid 4-(nitrooxy)butyl hydrochloride ester

To a solution of 1-(N-tert-butoxycarbonylamino methyl)cyclohexan acetic acid 4-(nitrooxy)butyl ester (0.8 g, 2.06 mmoles) in ethyl acetate (5 ml), a HCl 1N solution in ethyl acetate (20ml) is added. The reaction mixture is left for 3 hours at room temperature then is treated with n-hexane. The precipitate is filtered and dried under vacuum. 0.45 g of the expected compound are obtained as a white solid having m.p. = 80.3°-81.3°C

^{1}H-NMR (DMSO) ppm: 8.23 (2H, s); 4.58 (2H, t), 4.09 (2H, t); 2.92 (2H, s); 2.56 (2H, s); 1.74 (4H, m); 1.44 (10H, m).

EXAMPLE 3

Synthesis of the 1-(aminomethyl)cyclohexan acetic acid 3-(nitrooxymethyl)phenyl hydrochloride ester (XVI)



A) Synthesis of 3-(bromomethyl)phenol

To a solution of 3-hydroxybenzyl alcohol (4 g, 32.2 mmoles) in methylene chloride (250 ml), cooled at 0°C, tetrabromomethane (12.82 g, 38.6 mmoles) and triphenylphosphine (12.67 g, 48.3 mmoles) are added. The mixture is kept under stirring at 0°C for 10 minutes, then evaporated at reduced pressure. The crude product is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 8/2. 3.5 g of the expected compound are obtained.

B) Synthesis of the 1-(N-tert-butoxycarbonylamino methyl)cyclohexan acetic acid 3-(bromomethyl) phenyl ester

To a solution of 1-(N-tert-butoxycarbonylamino methyl)cyclohexan acetic acid (2.6 g, 9.7 mmoles) in chloroform (200 ml) and N,N-dimethylformamide (2 ml), 4-(bromomethyl)phenol (2 g, 10.7 mmoli), dicyclohexylcarbodiimide (4 g, 19.7 mmoles) and N,N-dimethylaminopyridine (24 mg, 0.20 mmoles) are added. The reaction mixture is left at room temperature for 4 hours under stirring, filtered and evaporated at reduced pressure. The obtained residue is treated with ethyl acetate and washed with water. The organic phase is dried with sodium sulphate and evaporated at reduced pressure. The obtained residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 9/1. 1.4 g of the compound are obtained as an oil.

C) Syynthesis of the 1-(N-tert-butoxycarbonylamino methyl)cyclohexan acetic acid 3-(nitrooxymethyl)phenyl ester

To a solution of 1-(N-tert-butoxycarbonylamino methyl)cyclohexan acetic acid 3-(bromomethyl) phenyl ester (1.4 g, 3.18 mmoles) in acetonitrile (300 ml) silver nitrate (1 g, 6.36 mmoles) is added. The reaction mixture is heated at 50°C for 4 hours sheltered from light. The formed salt is removed by filtration and the solution is evaporated at reduced pressure. The obtained residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 8/2. 0.75 g of the expected compound are obtained as an oil.

D) Synthesis of the 1-(aminomethyl)cyclohexan acetic acid 3-(nitrooxymethyl)phenyl hydrochloride ester

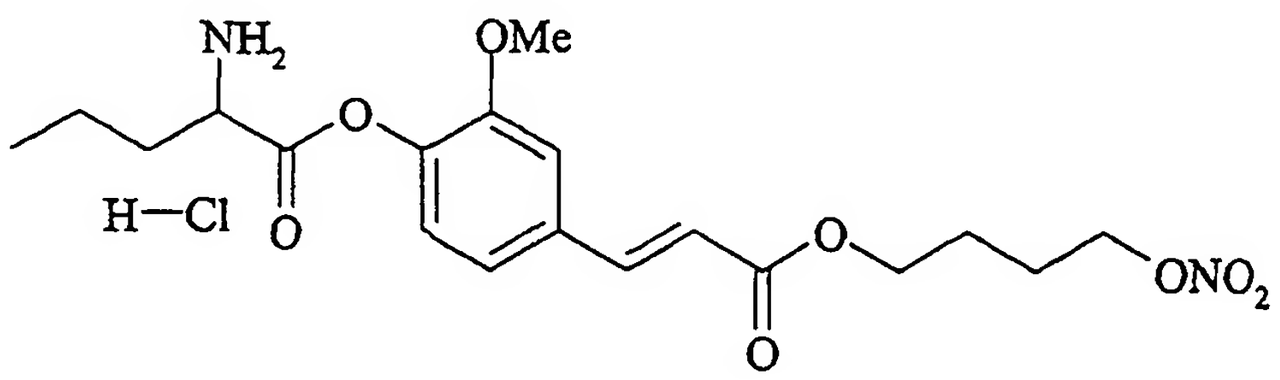
To a solution of 1-(N-tert-butoxycarbonylamino methyl)cyclohexan acetic acid 3-(nitrooxymethyl)phenyl ester

(0.75 g, 1.8 mmoles) in ethyl acetate (5 ml), a HCl 1N solution in ethyl acetate (18 ml) is added. The reaction mixture is left for 15 minutes at room temperature, then it is treated with n-hexane. The precipitate is filtered and dried under vacuum. 0.45 g of the expected compound are obtained as a white solid having m.p. = 106°-108°C.

^{1}H-NMR (DMSO) ppm: 8.16 (3H, m); 7.52 (1H, t); 7.44 (1H,d); 7.34 (1H, s), 7.28 (1H, d); 5.65 (2H, s), 3.03 (2H, m); 2.86 (2H, s); 1.55 (10H, m).

EXAMPLE 4

Synthesis of the 2-aminopentanoic acid 2-methoxy-4-[(1E)-3-[4-(nitrooxy) butoxy]-3-oxy-1-propenyl]phenyl hydrochloride ester



A) Synthesis of the 1-(N-tert-butoxycarbonylamino) pentanoic acid

To a solution of 2-aminopentanoic acid (4 g, 34.14 mmoles) in dioxane (40 ml) and water (75 ml), triethylamine (9.5 ml, 68.29 mmoles) and di-tert-butyldicarbonate (8.94 g, 49.97 mmoles) are added. The reaction mixture is left at room temperature, under stirring for 17 hours. After having cooled the solution at 0°C, it is brought to pH = 2 with HCl at 5%. It is extracted with ethyl acetate, the joined organic phases are washed with water and dried with sodium sulphate.

The solvent is evaporated at reduced pressure to give the compound as an yellow oil which is used without further purification.

B) Synthesis of 2-methoxy-4-[(1E)-3-[4-(bromo)butoxy]-3-oxy-1-propenyl]phenol

To a solution of ferulic acid (11.6 g, 59.7 mmoles) in tetrahydrofuran (400 ml), tetrabromomethane (39.62 g, 119.47 mmoles) and triphenylphosphine (31.34 g, 119.47 mmoles) are added. The obtained mixture is kept under stirring at room temperature for 5 hours, filtered and evaporated at reduced pressure. The obtained crude compound is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 7/3. 8 g of the expected compound are obtained as a yellow solid having m.p. = 86°-89°C.

C) Synthesis of 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl]phenol

To a solution of 2-methoxy-4-[(1E)-3-[4-(bromo) butoxy]-3-oxy-1-propenyl]phenol (8 g, 24.3 mmoles) in acetonitrile (500 ml) silver nitrate (12.25 g, 72.9 mmoles) is added. The reaction mixture is heated at 40°C for 12 hours sheltered from light. The formed salt is removed by filtration and the solution is evaporated at reduced pressure. The obtained residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 75/25. 4 g of the expected compound are obtained as a yellow solid having m.p. = 65°-68°C.

C) Synthesis of the 2-(N-tert-butoxycarbonylamino) pentanoic acid 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl]phenyl ester

To a solution of 2-(N-tert-butoxycarbonylamino) pentanoic acid (0.5 g, 2.3 mmoles) in chloroform (12 ml), 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl]phenol (0.86 g, 2.76 mmoles), dicyclohexylcarbodiimide (0.52 g, 2.53 mmoles) and N,N-dimethylaminopyridine (0.03 g, 0.23 mmoles) are added. The reaction mixture is left at room temperature for 1 hour under stirring, filtered and evaporated at reduced pressure. The obtained residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 75/25. 0.5 g of the expected compound are obtained as an oil. Yield 43%.

D) Synthesis of the 2-aminopentanoic acid 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl]phenyl hydrocloride ester

To a solution of 2-(N-tert-butoxycarbonylamino) pentanoic acid 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxy-1-propenyl]phenyl ester (0.28 g, 0.548 mmoles) in ethyl acetate (7 ml), a HCl solution in ethyl acetate (6.8 N, 0.700 ml) is added. The reaction mixture is left 3 hours at room temperature. The precipitate is filtered and dried under vacuum. 0.1 g of the expected compound are obtained as a white solid.

^{1}H-NMR (DMSO) ppm: 8.75 (3H, m); 7.62 (1H, d); 7.58 (1H, s); 7.3 (1H, d); 7.2 (1H, d); 6.72 (1H, d); 4.57 (2H, t), 4.26 (1H, t); 4.18 (2H, t); 3.82 (3H, s); 1.95 (2H, m); 1.75 (4H, m); 1.45 (2H, m); 0.98 (3H, m).

EXAMPLE F1

Evaluation of the analgesic activity of the invention compounds by the "paw-licking" test

Four groups of Swiss male mice (20-25 g, Charles River) each formed by 10 animals, received by intraperitoneal

injection Gabapentin (90 mg/kg) or the compound of formula (XVI) (Example 3), called NO-Gabapentin at the doses of 50 mg/kg, in a saline solution. The control group received the same volume of saline solution. One hour after the administration of the compound solutions, formalin (10 µl) was injected in the paw. In the 15 minutes subsequent to formalin administration, for each animal, the number of times wherein it licked its paw was counted. The analysis was carried out "in blind".

The results reported in Table 1 are expressed as percentage ratio between the number of times wherein the "paw-licking" was observed in the treated animals to that of the control group.

The results show that the NO-gabapentin is more active than the starting drug in inhibiting the "paw-licking".

EXAMPLE F2

Evaluation of the analgesic activity of the drugs used in the chronic (neuropathic) pain treatment combined with a nitric oxide-donor drug.

Wistar adult rats weighing about 200 grams were used, in the experimental model described by Bennett GJ, Xie YK, Pain 1988, 33(1): 87-107. The pain response (withdrawal latencies) is determined 14 days after the ligature of the right sciatic nerve. The results obtained are reported in Table 2 and have been expressed as a percentage ratio of the difference between the response from the intact paw and from the injured paw, to the response of the control animals, that have been injected with the carrier and undergone the nerve ligature. The groups were of 10 rats each. The animals in each of the treated groups received, respectively, the following drugs at the

indicated doses:

- clomipramine 10 mg/kg i.p.,
- 2-acetylsalicylic acid (3-nitrooxymethyl)phenyl ester (ASA-NO) 100 mg/Kg p.o.
- clomipramine + NO-ASA at the above indicated doses.

NO-ASA was prepared as described in patent application WO 97/16405.

The results are reported in Table 2 and show that the mixture of the analgesic drug with the nitrooxyderivative synergically increases the analgesic effect.

EXAMPLE F3

Acute toxicity of gabapentin and NO-gabapentin in the diabetic animal

In 50 Wistar adult male rats weighing 165-190 g, diabetes was induced by injection i.v. of a single dose of streptozocin (65 mg/kg in 1 ml/kg buffer citrate at pH 4.5).

After one week the animals were distributed in three groups of 10 rats each and treated per os for three days with daily doses of 100 mg/kg of gabapentin and NO-gabapentin. The controls were treated only with streptozocin.

Death rate was monitored for 7 days from the last treatment.

The results are indicated in Table 3.

The Table shows that death rate in the diabetic animals administered with NO-gabapentin is less than half with respect to the animals treated with gabapentin.

EXAMPLE F 4

In this experiment the effect of acute administration of NO-gabapentin was assessed and compared with that of the

precursor drug, gabapentin, in a rat model of neuropathic pain.

In 6 groups of female Sprague-Dawley rats weighting 200 g, photochemically-induced ischemic spinal cord injury was produced according to methods described by Xu et al. Pain, 1992, 48, 279-290. Spinally-injured rats developed a chronic pain syndrome, including marked mechanical and cold allodynia. The rats were injured 3-6 months before the beginning of the experiment.

Each group of rats was i.p. treated, respectively, with one of the following compounds using one of the above indicated doses:

- NO-gabapentin at doses of 20 mg/Kg (55 µmole/Kg), or 60 mg/Kg (167,21 µmole/Kg), or 100 mg/kg (278,7 µmole/Kg), dissolved in saline, single dose, i. p.);
- gabapentin at doses of 30 mg/Kg (175 µmole/Kg) or 100 mg/kg (584 µmole/Kg), dissolved in saline, single dose i. p.);
- the control group received the vehicle;

The response to cold was tested by spray-applying ethyl chloride on the shaved allodynic skin area at time 0(i.p. injection) and then at 30', 120' and 240'. The response was evaluated according to the following score:

0 = no response;

1 = localized response (skin twitch and contraction), no vocalization;

2 = transient vocalization, moderate struggle;

3 = sustained vocalization and aggression.

The results are reported in Table 4 and show that acute administration of NO-gabapentin alleviated in a dose-dependent way cold allodynia.

The effect of the compounds on motor activity was also evaluated using a combined testing system as described in Hao, J.X. and Xu, X. J., Pain, 1996, 66, 279-286.

Table 5 reports the results on motor performance of the same groups of rats i.p. injected.

When the score found in the treated group is comparable to that of the control groups it means that the animals of the treated group are not impaired as to motor activity and are not sedated.

Comments on Tables 4 and 5

Table 4 shows that the effect of NO-gabapentin was significant and lasted 120 min with the dose of 60 mg/kg (167,21 µmole/Kg) and 240 min with the dose of 100 mg/kg (278,7 µmole/Kg). The vehicle produced no effect. Gabapentin at the dose of 30 mg/Kg (175 µmole/Kg) did not produce a significant antiallodynic effect (Table 4). At the dose of 100 mg/kg (584 µmole/Kg), induced motor impairment and sedation (Table 5), which made it difficult to evaluate its anti-allodynic effect.

EXAMPLE F 5

In this experiment the effect of repeated administration of NO-gabapentin was assessed and compared with that of the precursor drug gabapentin in a rat model of neuropathic pain. In 4 groups of female Sprague-Dawley rats weighting 200 g photochemically-induced ischemic spinal cord injury was produced according to methods describe by Xu et al. Pain, 1992, 48, 279-290.